DETECTION OF SEASONAL PRATYLENCHUS BRACHYURUS NEMATODE POPULATIONS

By

PHILLIP WADE PRATT

Bachelor of Science

Oklahoma State University

Stillwater, Oklahoma

1971

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May, 1977

DETECTION OF SEASONAL PRATYLENCHUS BRACHYURUS NEMATODE POPULATIONS

Thesis Approved:

Thesis Adviser

Dean of the Graduate College

ACKNOWLEDGMENTS

The author wishes to express his appreciation and gratitude to Dr. R. V. Sturgeon Jr., for his confidence and guidance throughout the course of this study. The author also takes this opportunity to express his appreciation to his committee members: Dr. Charles C. Russell and Dr. Dallas F. Wadsworth.

To my wife, Mary Carole, for her encouragement this thesis is dedicated.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The assay for root-lesion nematode, Pratylenchus brachyurus, populations in soil of Oklahoma peanut fields has become an important aspect of the advisory services offered to Oklahoma peanut growers by the Oklahoma State University Extension Service.

The farmers are becoming more aware of plant parasitic nematodes and the damage they cause. There is an urgent need for development of more efficient sampling and extracting techniques to aid the advisory personnel in making accurate nematode control recommendations and in supporting judicious chemical use in pest management programs.

Steps in assay procedure for advisory purposes generally involve sampling, handling, laboratory analyses, and interpreting the results for the purpose of making control suggestions. The root-lesion nematode, P. brachyurus, is found on many crops and is one of the most destructive pests that occurs on peanuts in Oklahoma. This nematode is capable of causing both a reduction in yield and quality of the peanuts produced in infested fields (12). However, it is difficult to accurately assess the P. brachyurus nematode population in a field on the basis of soil samples taken during the months of December through June. The objectives of this study were to determine the location of P. brachyurus nematode populations throughout the year and to find a more efficient means of sampling. Also, the study was designed to determine if

there was migration of the nematode population during the summer months. Various laboratory procedures and soil analysis methods were tested and evaluated to determine the most accurate method of detecting the nematodes present in the soil samples taken. An accurate procedure of determining the P. brachyurus nematode population during the late winter and early spring months is needed by advisory personnel to predict crop season populations, thus aiding the growers in making decisions on the selection of crops, land use, application of nematicides, and other practices that might be employed to reduce nematode damage. It is hoped that this study will provide information needed to develop procedures to predict the occurrence of the Pratylenchus brachyurus nematode populations.

CHAPTER II

LITERATURE REVIEW

Tylenchus penetrans was described in 1880 by deMann, who was later given credit for being the first to describe a root-lesion nematode, Pratylenchus sp. (32). The symptoms caused on the roots and underground fruits by Pratylenchus spp. are generally described as necrotic dark lesions. These lesions cause a reduction in the quality of the underground fruit and provide an excellent means of entry for fungal pathogens that may be present in the soil (2, 12, 26). The distribution of Pratylenchus spp. is world wide and Pratylenchus brachyurus has a very wide host range (33,35, 39).

The damage to peanuts being attacked by Pratylenchus nematode spp. was first reported by Steiner in 1945, and in following years the root-lesion nematode reported to attack peanuts was described as Pratylenchus leiocephalus (34). Good described P. leiocephalus as an endoparasitic nematode that was difficult to recover from peanuts and, for a few years, populations were thought to be reduced under a peanut cropping program (11). In 1953 Sher and Allen (32) revised the genus Pratylenchus (Nematode: Tylenchidae) and made the nematode described by Steiner as Pratylenchus leiocephalus, a synonym of Pratylenchus brachyurus.

Pratylenchus bracyurus nematode was first reported by Struble et. al. (36) on peanuts in Oklahoma on September 18, 1959. Ten P. brachyurus nematode larvae were extracted from peanut pods collected from a

field near Binger, Oklahoma in Caddo county. Upon resampling the field in the spring of 1960, no P. brachyurus could be recovered from the soil samples collected (24). P. brachyurus nematode was not reported again in Oklahoma until October, 1962 and it was again found on peanuts from a southern Oklahoma peanut field located near Antlers, Oklahoma in Pushmataha county. Since finding populations of P. brachyurus nematode in 1962, it has been found in many fields throughout southern Oklahoma's peanut producing area. At this time, it is impossible to estimate the full extent of the P. brachyurus nematode infestation in Oklahoma.

Good, Robertson, and Thompson (11) in 1954 reported that the nematode P. leiocephalus would build up to a higher infestation level on corn than on peanuts and suggested that peanuts might even be used in a crop rotation with corn to reduce P. leiocephalus nematode populations. However, there was no record of these workers processing the pods and pegs of the peanut plants for making nematode counts. Endo, (9) working at North Carolina State University, tested thirty plant species as host for P. brachyurus nematode and found that populations became very high on both corn and peanuts. Early studies on P. brachyurus nematode (11) dynamics have been interpreted that peanuts could be used to lower P. brachyurus nematode infestation in the soil. Later studies by Good, Boyle, and Hammons (12) indicated this was an incorrect conclusion because the earlier studies had been based on the number of nematodes recovered from soil and roots. To obtain a more accurate sampling of the P. brachyurus nematode population, the pods, or shells had to be involved in the extraction technique because the reproduction in shell tissue is six to eight times greater than in an equal amount of root tissue. This may be the reason that earlier researchers found low soil

and root populations of P. brachyurus nematodes when extraction techniques were performed on the soil in association with peanut plants. Research studies on the pods, shells, and pegs of peanut plants have revealed that these structures are the main source of inoculum for the nematode P. brachyurus (10, 12, 13, 14, 21, 22) and provide protection for the root-lesion nematode from chemical fumigation (14, 22).

Research involving the nematode species P. brachyurus relies on four or five main methods for extracting the nematodes from the soil. One of the most widely used, especially in early studies, is the Baermann funnel (1). Another method sometimes used is the Christie and Perry technique (5), and a technique described by Seinhorst (31) is perhaps the most accurate for detailed census work. Centrifuge techniques describes in 1955 by Caveness and Jensen (4) and modified by several workers (15,20) have been used to some extent for soil extractions. Extraction of P. brachyurus nematode from roots, pods, and pegs is generally achieved by two techniques or slight modification of these two basic procedures. A method termed the Waring blender method was developed by Taylor (37) and an incubation method which gives excellent results was developed by Young in 1954 (43). Even though all of these techniques have been employed, it still remains difficult to extract P. brachyurus nematodes from soil and/or peanut pods collected from peanut fields in Oklahoma and Texas with known P. brachyurus nematode infestations during late winter and spring (38). Peanut shells and pods collected from Oklahoma and Texas peanut fields are an ineffective means of detecting P. brachyurus, especially if the pods and pegs are found below the soil surface and have started the decay process (29, 38). Russell and Shackelford found it difficult to detect and extract P.

brachyurus nematode from pods, pegs, and soil that were collected during the winter and spring of 1969 from fields with known P. brachyurus nematode infestations (28, 29).

Willis (42) found that Pratylenchus penetrans nematode reproduction was greater at pH levels of 5.2 and 6.4. Collins and Rodriguez-Kabana (6) obtained similar results in their work with Pratylenchus spp. Koen (18) reported a study in which P. brachyurus nematode larvae were placed in pH values of 1, 3, 5, 7, and 7.3 (tap water). There were no significant differences of larvae left alive in pH levels of 5, 7, and 7.3. The pH of soil of the peanut fields in Oklahoma range between 4.0 and 8.0, with the majority of the fields in the 5.8 to 6.8 range (40). This pH range is well within the range of P. brachyurus nematode activity.

Temperature effects on Pratylenchus nematode spp. have been studied by several researchers. Soil populations of Pratylenchus penetrans nematodes were reported to reach their highest levels in the rhizosphere of strawberry roots in June and root populations reached their highest number in July. The lowest soil populations were found in January when the soil temperatures reached freezing or near freezing temperatures (7). Nevertheless, it was reported by Dunn (8) that the nematode P. penetrans was capable of embryonic development and maturation at winter soil temperatures (0 to 3.5 C). It appears that various Pratylenchus nematode spp. have different soil temperature tolerances. Patterson and Bergeson (25) found that 30 C was more favorable to P. penetrans nematode reproduction and activity than 15 or 22.5 C. Kerr and Vythilingam (17) working with the nematode P. loosi found the number of nematodes extracted from soil was greatly reduced when the soil temperature was 27 to 35 C. The number of nematodes in a sample was also found to

reduced in soil stored at temperatures of 31 to 35 C. The temperatures that caused reduction in number during extraction did not cause a reduction in numbers during storage, but the mobility of the nematodes was thought to be affected. Feldmesser and Rebois (10) found that Pratylenchus brachyurus nematodes in roots could survive 2.9 hours per day for 13 days at 37.8 C. They also found that 25 to 50 percent of P. brachyurus nematode present in soil populations have the ability to survive daily exposure periods of 0.6 and 1.2 hours at soil temperatures as high as 43.3 C. This ability to survive high soil temperatures was not lost even at low soil moisture levels of 0.5%, 0.3% and 0 percent. This indicates that P. brachyurus nematode could be spread in dried debris such as pods, pegs, and roots remaining in the field after harvest. Soil temperatures at 15 C to 20 C were found to be most favorable for P. brachyurus nematode by researchers in Georgia (2).

Soil moisture is also an important factor in the activity of P. brachyurus nematode. Kable and Mai (16) found that Pratylenchus penetrans nematode rate of population increase is greatest when soil moisture is at a moderate level and the least amount of population increase occurs at very low or very high soil moisture levels. Meagher (19) found in a study involving the nematode species Heterodera avanae, Pratylenchus minyus, and Tylenchorhynchus brevidens that survival of the nematode larvae was very low when soil moisture was at field capacity. When soil in the greenhouse was alternated through a wet and dry cycle larval emergence was increased. Meagher also reported that P. minyus nematode had the ability to survive in air dried soil, but he did not know what the mechanism was for survival. He thought the nematode species studied in this test were dependent on both soil moisture and soil temperature before they became active in the soil. Pratylenchus

brachyurus nematode was found by Brodie and Quattlebaum (3) to produce its highest numbers when soil moisture was thirty percent. Radewald and Takeshita (27) reported that P. brachyurus nematodes failed to survive after four months of dessication in potted field soil with no water or host plants. However, when pots of the same field soil were maintained for the same time period and watered regularly, P. brachyurus nematode survived.

Variation in the number of P. brachyurus nematodes found at different depths in the soil has been reported by several workers. In work done in South Africa (18), involving a loamy sand soil type, the highest number of P. brachyurus nematode was found at a depth of 20 to 30 cm. Brodie (3) working in Georgia, reported the highest number of P. brachyurus nematode was found at a depth of 45 to 90 cm, and soil temperatures of 15 to 20 C. In Oklahoma studies conducted by Russell, Shackelford, and Morrison (28), it was found that P. brachyurus nematode populations, at sampling depths of 7.62, 15.24, 30.48, 45.72, and 60.96 cm, during June, July, and December were so low that they could have easily been missed with commonly used soil sampling procedures. Other observations made by these workers were that P. brachyurus nematode populations were distributed at soil sampling depths below those commonly suggested by advisory personnel, and that a 100 milliliter aliquot of soil was not a large enough soil sample to use for diagnostic purposes when the root-lesion nematode is the target species. Also, it was stated that the small feeder roots, often left in the field by the sampler or discarded in preference to larger roots, frequently contain the largest percentage of the recoverable P. brachyurus nematode population.

Wang and Bergeson (41) found that tomato roots infested with the

nematode Meloidogyne incognita, an endoparasite, exude more sugar than noninfested roots. The exudate from galled roots contained three sugars, twelve amino acids, and three organic acids. The healthy root exudate contained the same sugars, amino and organic acids found in the exudate from galled roots plus one additional sugar, three additional amino acids, and one additional organic acid. Monoson, et. al. (23) reported their work indicated that nematode trapping fungi produce a nematode attracting substance (NAS) which attracts nematodes (species unnamed) to the fungi. This is not the same as "nemin", the substance nematodes produce to stimulate nematode fungi in producing the trapping structures. They could not identify the substance which attracted the nematodes on agar to a droplet of NAS. They did not establish what substance or substances composed the NAS properties. They tested some known plant hormones and cyclic AMP and found that Indol-3 acetic acid, kinetin, and gibberellic acid displayed no attraction properties for the nematodes. Three amino acids were also tested for their NAS properties, and arginine, alanine, and tryosine demonstrated no nematode attraction qualities. At this time no work has been reported on the exudates of plant roots and their nematode attraction properties.

CHAPTER III

FIELD PRATYLENCHUS BRACHYURUS POPULATIONS IN ASSOCIATION WITH TIME AND DEPTH

The main objective of this study was to determine the location of the root-lesion nematode, P. brachyurus, population in certain soil horizons throughout the year.

Methods and Materials

Three fields on the Dee Keeton farm located in Marshall county near Willis, Oklahoma were selected for this study because a detailed history of a high P. brachyurus nematode infestation was available. This farm had been used as a test site for nematicide evaluation by the Plant Pathology Department, Oklahoma State University since 1969.

During this study, which was conducted in 1973, field number 1 was planted in rye and lespedeza from January to March and had not been planted in peanuts since 1970. April through July the field was planted in corn and in late August rye cover crop was planted.

Spanish peanuts were planted in field number 2 on May 21 following a rye cover crop maintained during the period of January through May 10. The peanuts were harvested on October 20, 1973. This field had been in a peanut-rye cover crop rotation since 1968. The thesis sampling area was located in a non-treated plot within the 1973 soil nematicide evaluation studies carried out by Oklahoma State University. The same area

has been used as nematode evaluation site in 1972, hence the area selected for this study could have received a nematode control treatment in 1972.

Field number 3 had a cropping history of Spanish peanuts with a winter cover crop of rye dating back to 1968 and was planted in cotton the years 1964 through 1967. During this study the field was planted to rye as a winter cover crop January through May 15 and planted to Spanish peanuts on May 20 and harvested on October 14. The field, including the sampling area, was treated with the nematicide O, O-Diethyl O - (P-(methylsulfinyl) Phenyl) Phosphorothidate, (Dasanit 15 G, 3 lbs. ai/a), applied at pegging time, and the same rate of nematicide was applied to this area in 1971 and 1972.

The sampling areas were located in 3 fields and consisted of 4 sampling sites in each field with soil samples taken at 5 levels at each site on 15 sampling dates. Samples were taken monthly January through April and August through December and bi-monthly during May, June, and July. The field sampling areas were 36.6 meters long and 3.7 meters wide. The 4 sampling sites within each area were selected at 0.92 meter intervals. This was done to prevent the removal of too many peanut plants from any one area during the sampling. The 4 sampling sites within each area were predetermined by numbering the sampling intervals and then randomly selecting from the odd numbered intervals only. When row crops were growing in the area the samples were taken from within the plant rows. During the time peanuts were not in the field, similar 0.92 meter intervals were maintained to keep the sampling sites within the rows. Hence, 4 rows were sampled on each of the sampling dates. (I) 0 to 7.62 cm , (II) 7.62 to 15.24 cm , (III) 15.24 to 22.86 cm ,

(IV) 22.86 to 30.48 cm , and (V) 30.48 to 38.10 cm. Thus, the study consisted of 3 plot areas. Each area contained 4 sampling sites which were sampled at 5 depth, resulting in 60 soil samples taken on 15 sampling dates.

The soil sampling procedure consisted of digging a hole approximately 51 cm in depth and 30 cm in diameter. Soil samples of approximately 950 milliliters were taken at each of the 5 sampling depths by removing soil at the various depths from the sides of the hole using a small garden trowel. Soil temperature readings were taken at each of the sampling depths by inserting a glass centigrade therometer into the side of the hole at each sampling depth. The soil was placed in a marked non-vented polyethylene bag and the samples were taken in a fiber glass insulated ice chest to prevent over exposure to the sun and for transport back to the Plant Diagnostic Laboratory at Oklahoma State University. Soil samples were processed for nematodes within 36 hours of sampling using a Modified Seinhorst Quick Extraction technique (30) and soil pH readings made at this time with a Coleman Model 28B Metrion III pH Meter on each soil sample. After 24 hours, the samples were decanted poured into a watch glass and identification and counts of the nematodes were made using a stereoscopic microscope.

Results and Discussion

Nine hundred soil samples were analyzed for the presence of P. brachyurus nematode from the 3 sampling areas. Samples analyzed from area 1 revealed no P. brachyurus nematodes throughout the sampling period. Therefore, this location will not be reported in the results of this study. The P. brachyurus nematodes recovered from the soil

samples taken on 15 sampling dates from field areas 2 and 3, through the period of January 1 to December 10, 1973, are presented in Tables I and II and depicted in graph-form in Figures 1 and 2.

During the months of December, January, February, March, April, and May the combined P. brachyurus nematode population recovered from the two upper sampling depths of 0 to 7.62 cm and 7.62 to 15.24 cm made up less than 18 percent of the total population found during this period, Table III. Higher population levels of P. brachyurus nematode were found to occur during these months primarily in the lower depths of 15.24 to 38.10 cm. At these depths, 83 to 93 percent of the population in area 3, and 0 to 100 percent of the total population in area 2 were found. This illustrates an interesting aspect of the field sampling study in that, during the months when cold temperatures occurred, populations were reduced to subdetectable levels in the upper sampling depths. However, at the lower sampling depths, 30.48 to 38.10 cm, the P. brachyurus nematode appeared to be slightly active and could be detected with limited success by standard soil sampling procedures. This leads one to believe that the surviving population at the 30.48 to 38.10 cm depth has some protection from the sudden changes in the soil temperature. Although the soil temperature at the lower depths is near to that recorded at the upper sampling depths (Figures 3 and 4), it is more likely to remain constant and not fluctuate with changes of air temperature. It is possible that the active P. brachyurus nematode population at the lower sampling depths gives rise to the population that occurs later in the season in the upper horizons of the soil. However, this is not indicated by the data to be a physical migration upward in the soil and could be influenced by favorable conditions for reproduction

TABLE I

PER CENT OF TOTAL *P. BRACHYURUS* NEMATODE POPULATION RECOVERED BY DEPTH PER MONTH PER 100 MILLILITERS ALLIQUOT OF SOIL FROM AREA 2

Sampling Depth	1973 Date of Sampling															% ($\bar{X}$ Total Count)
	Jan	Feb	Mar	Apr	May 7	May 21	Jun 12	Jun 24	Jul 15	Jul 31	Aug	Sep	Oct	Nov	Dec	
0 - 7.62 cm	0(0)*	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	100(1)	100(3)	0(0)	0(0)	0(0)	0(0)	0(0)	9(4)**
7.62 - 15.24	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	100(1)	20(1)	25(2)	4(1)	0(0)	11(5)
15.24 - 22.86	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	100(1)	100(1)	0(0)	0(0)	0(0)	20(1)	13(1)	18(4)	0(0)	17(8)
22.86 - 30.48	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	60(3)	62(5)	68(5)	0(0)	50(23)
30.48 - 38.10	0(0)	100(1)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	9(2)	100(3)	13(6)
% ($\bar{X}$ total count/mo)	0(0)***	2.2(1)	0(0)	0(0)	0(0)	0(0)	2.2(1)	2.2(1)	2.2(1)	6.5(3)	2.2(1)	11(5)	17(8)	48(22)	6.5(3)	

* = % of total monthly counts (Means of monthly total of *P. brachyurus* nematodes found at each sampling depth in a 100 ml. alliquot of soil).
** = % of total yearly counts (Means of yearly total of *P. brachyurus* nematodes recovered at each sampling depth in a 100 ml. alliquot of soil).
*** = % of total of yearly counts (Means of yearly total of *P. brachyurus* nematodes recovered by month in a 100 ml. alliquot of soil).

TABLE II

PER CENT OF TOTAL P. BRACHYURUS NEMATODE POPULATION RECOVERED BY DEPTH PER MONTH PER 100 MILLILITERS ALLIQUOT OF SOIL FROM AREA 3

Sampling Depth	Jan	Feb	Mar	Apr	May 7	May 21	Jun 12	Jun 24	Jul 15	Jul 31	Aug	Sep	Oct	Nov	Dec	%($\overline{X}$ Total Count)
0 - 7.62 cm	20(1)*	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	25(1)	0(0)	18(2)	16(4)	0(0)	17(1)	10(9)**
7.62 - 15.24	0(0)	0(0)	0(0)	29(2)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	9(1)	32(8)	33(3)	0(0)	15(14)
15.24 - 22.86	60(3)	100(1)	83(5)	14(1)	47(7)	100(1)	0(0)	0(0)	0(0)	50(2)	0(0)	18(2)	28(7)	56(5)	33(2)	40(36)
22.86 - 30.48	0(0)	0(0)	0(0)	43(3)	40(6)	0(0)	0(0)	0(0)	0(0)	25(1)	0(0)	9(1)	20(5)	0(0)	33(2)	20(18)
30.48 - 38.10	20(1)	0(0)	17(1)	14(1)	13(2)	0(0)	0(0)	0(0)	100(1)	0(0)	0(0)	46(5)	4(1)	11(1)	17(1)	15(14)
%($\overline{X}$ total count/mo)	5(5)***	1(1)	7(6)	8(7)	16(15)	1(1)	0(0)	0(0)	1(1)	4(4)	0(0)	12(11)	28(25)	10(9)	7(6)	

* = % of total monthly counts (Means of monthly total of P. brachyurus nematodes found at each sampling depth in a 100 ml. alliquot of soil).
** = % of total yearly counts (Means of yearly total of P. brachyurus nematodes recovered at each sampling depth in a 100 ml. alliquot of soil).
*** = % of total yearly counts (Means of yearly total of P. brachyurus nematodes recovered by month in a 100 ml. alliquot of soil).

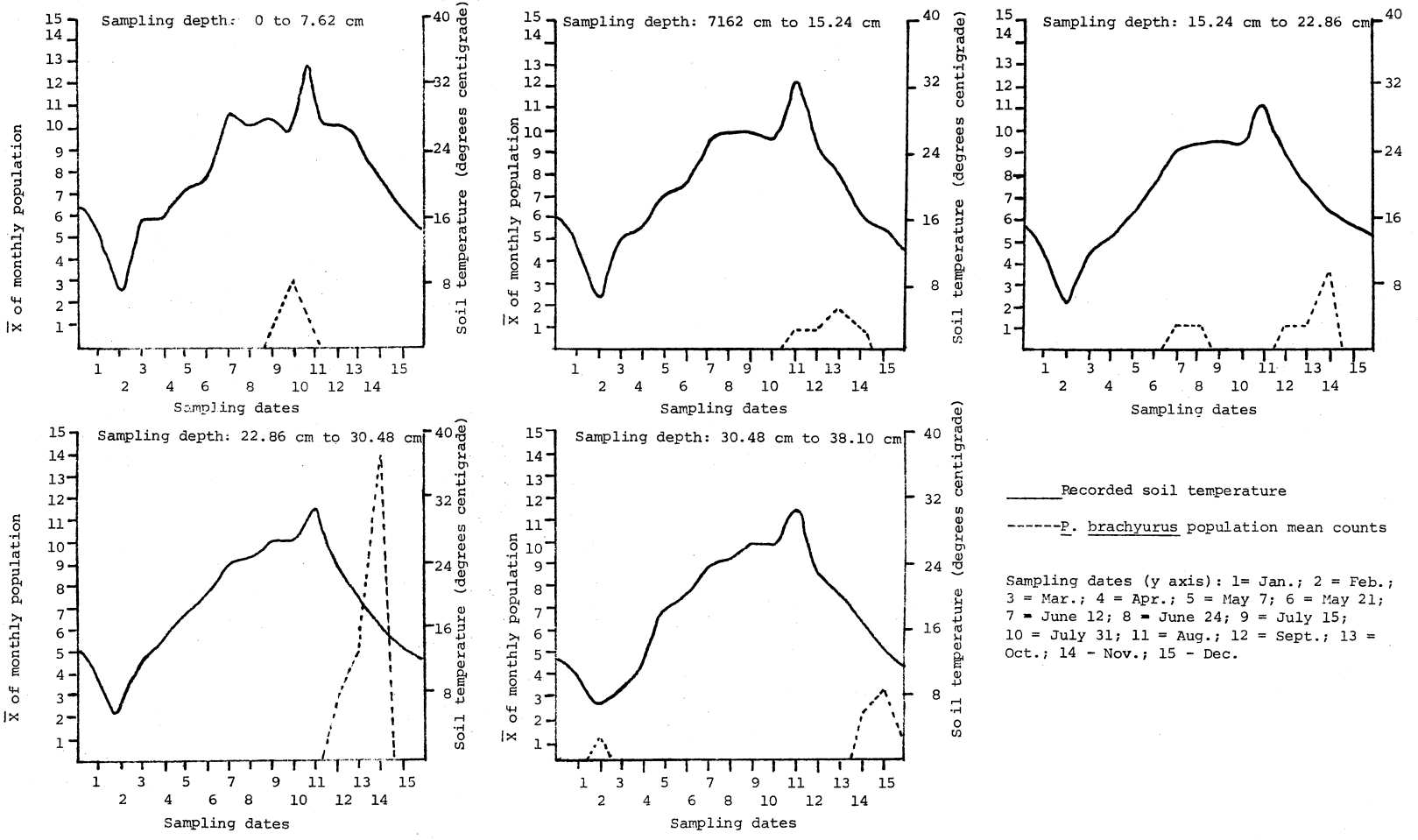

Figure 1. P. brachyurus Nematode $\bar{X}$ Monthly Population by Sampling Depth in Correlation to Sampling Date and Soil Temperature. Area 2

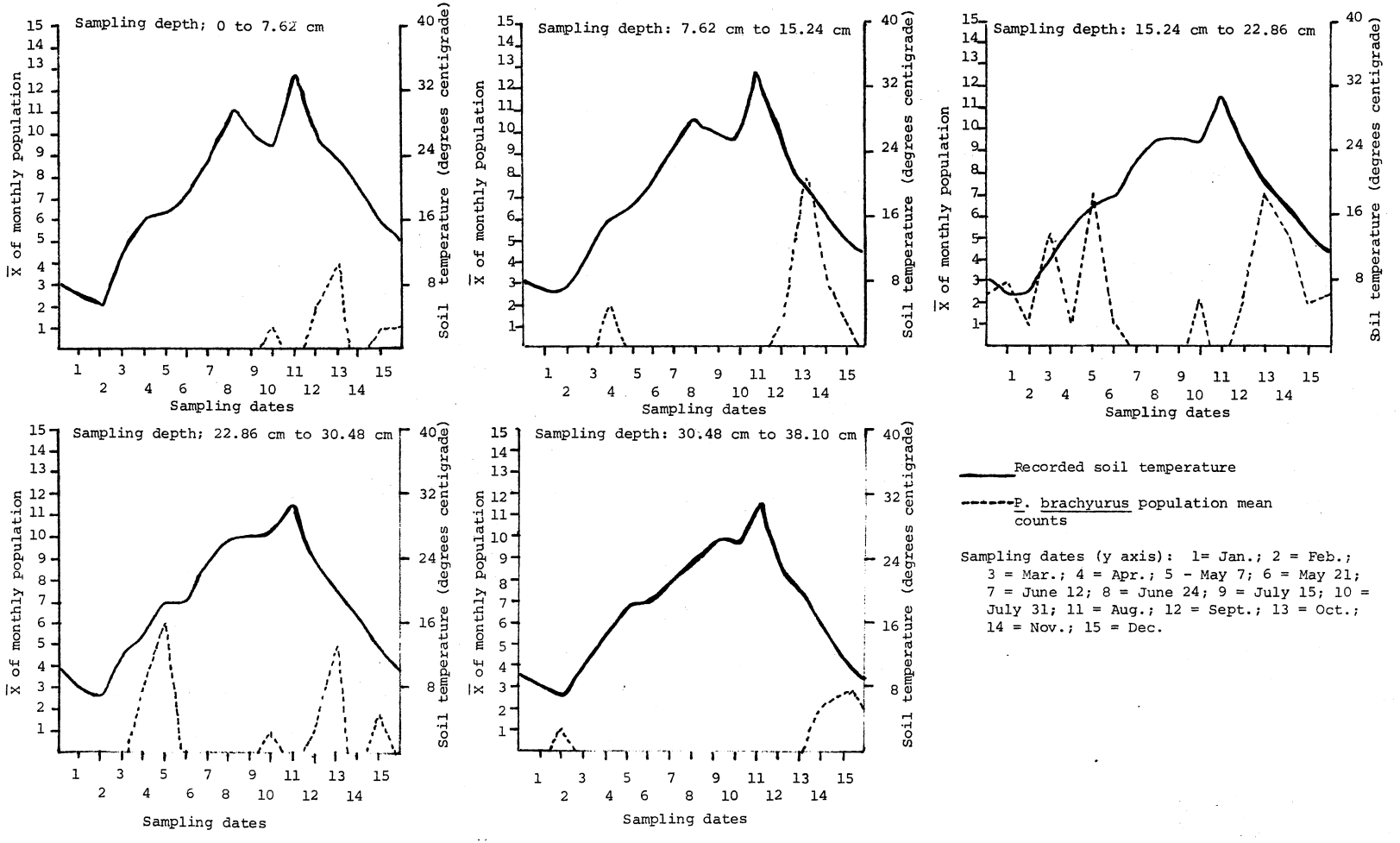

Figure 2. P. brachyurus Nematode $\bar{X}$ Monthly Population by Sampling Depth in Correlation to Sampling Date and Soil Temperature. Area 3

TABLE III

COMPARISON OF P. BRACHYURUS NEMATODE POPULATIONS FOUND WITHIN TWO SOIL SAMPLING LEVELS AT QUARTERLY PERIODS OF THE YEAR

	Sampling Area 2		Sampling Area 3	
	Top Level Percent*	Lower Level Percent**	Top Level Percent*	Lower Level Percent**
Dec.*** Jan. 1st Feb. qt.	0	100	17	83
Mar. Apr. 2nd May qt.	0	0	7	93
Jun. Jul. 3rd Aug. qt.	71	29	20	80
Sep. Oct. 4th Nov. qt.	11	89	40	60

* = Percent of P. brachyurus nematodes recovered from upper 2 sampling depths (0 - 7.62 cm and 7.62 - 15.24 cm).

** = Percent of P. brachyurus nematodes recovered from lower 3 sampling depths (15.24 - 22.86 cm, 22.86 - 30.38 cm, and 30.48 - 38.10 cm).

*** = Months of the year were grouped into quarters according to their air temperatures.

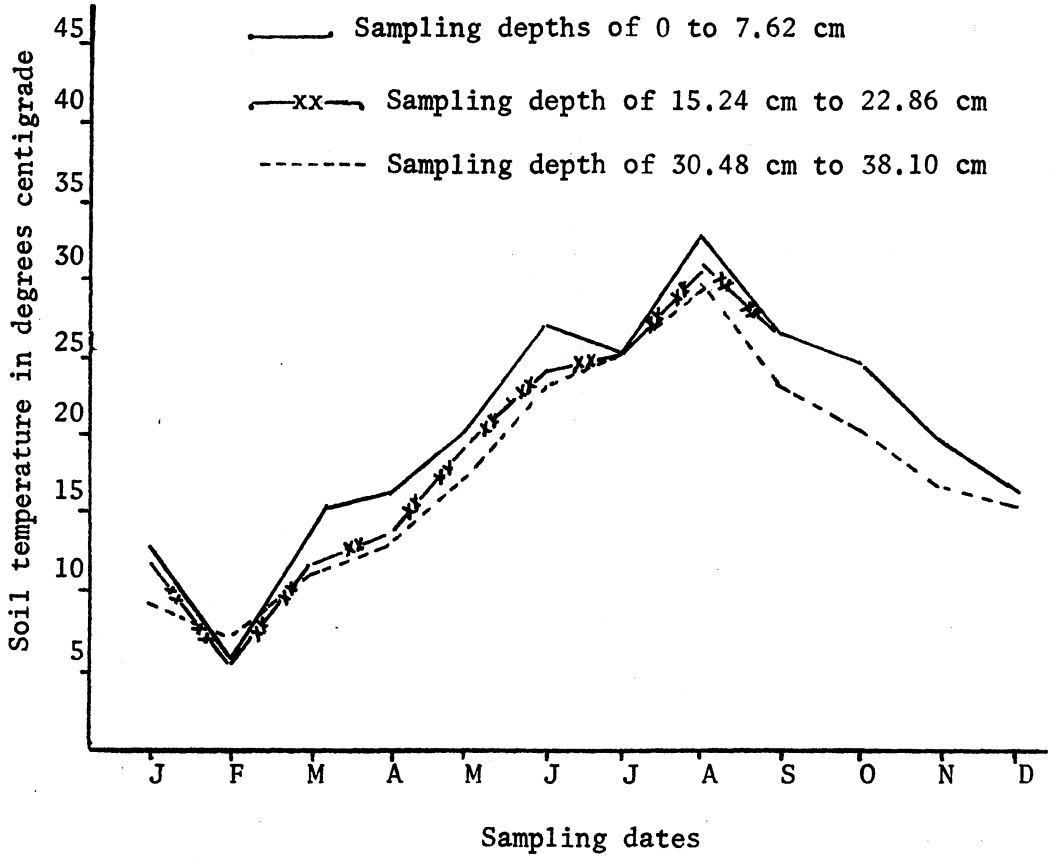

Figure 3. Soil Temperatures Recorded Throughout the Season at Three Different Sampling Depths, Samling Area Number 2

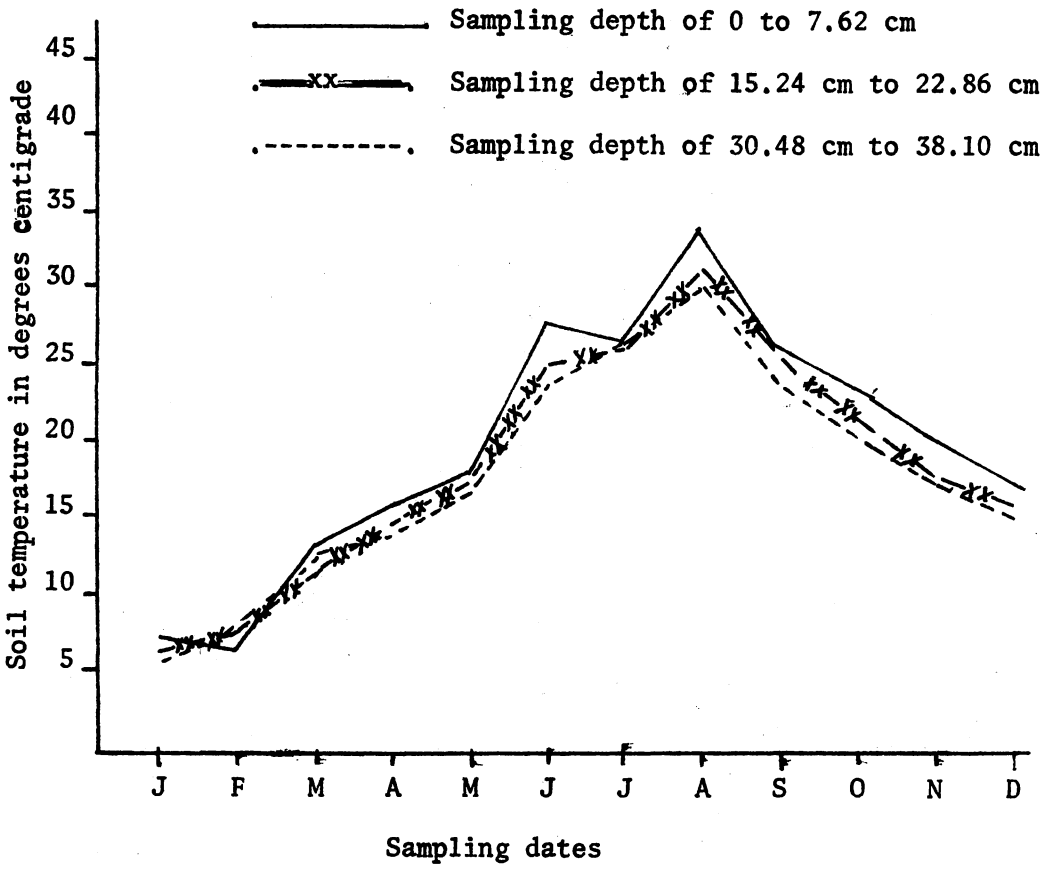

Figure 4. Soil Temperatures Recorded Throughout the Season at Three Different Sampling Depths, Sampling Area Number 3

found at these soil levels. The data can be interpreted as showing this phenomenon occurring at sampling area 2 (Tables I and II) as the season becomes warmer a larger percentage of the total monthly population occurs in the upper sampling depths. Then in the fall and early winter, as the soil temperature becomes colder, the largest percentage of the total monthly population begins to occur at the lower levels in the soil.

During the warmer season of the year, June, July, and August the soil temperatures ranged from 23.0 to 34.1 C at the various sampling depths and the nematode population was quite variable. Throughout June, July, and August 71 percent of the population was found in the top sampling levels in area 2, which received no nematicide treatment, as compared to 20 percent of the total population found in the upper levels in area 3 which received a nematicide treatment (Table III).

When seasonal comparisons were made of the P. brachyurus nematode population recovered in area 2, from the 2 upper sampling levels and lower 3 sampling levels, the greatest seasonal populations were recovered during June, July, and August in the upper levels and September, October, and November in the lower sampling levels. Of all the P. brachyurus nematodes found in the upper 2 sampling levels in area 2, 56 percent were found during July thru August, 44 percent during September thru November, and none December thru June (Table IV). In the 3 lower sampling levels in area 2, 84 percent of the P. brachyurus nematode population was recovered during September, October, and November, 11 percent during December, January, and February, and 5 percent during June, July, and August. Populations of P. brachyurus nematode were not recovered from the soil at any level during March, April, and May Table IV).

TABLE IV

SEASONAL PERCENT OF YEARS TOTAL POPULATION OF P. BRACHYURUS NEMATODE RECOVERED AT 2 SAMPLING LEVELS

	Sampling Area 2		Sampling Area 3	
	Upper Level Percent***	Lower Level Percent****	Upper Level Percent	Lower Level Percent
Dec. * Jan. 1st. Feb. qtr.	0 **	11	9	14
Mar. Apr. 2nd. May qtr.	0	0	9	40
June July 3rd. Aug. qtr.	56	5	4	6
Sept. Oct. 4th. Nov. qtr.	44	84	78	40

* = Month of the year grouped into quarters according to their air temperatures

** = Percent of the yearly total number of P. brachyurus recovered during the month listed in the left column.

*** = Percent of the year's total P. brachyurus nematode population recovered during the quarter from the upper two sampling depths (0 - 7.62 cm and 7.62 - 15.24 cm).

**** = Percent of the year's total P. brachyurus nematode population recovered during the quarter from the lower three sampling depth (15.24 - 22.86 cm, 22.86 - 30.48 cm, and 30.48 - 38.10 cm).

During the spring and summer months the P. brachyurus nematode population, when it can be detected, appears to be concentrated at the depths where the largest amount of peanut roots, pods, and pegs are located (Tables I and II). The P. brachyurus nematode population at the number 3 sampling area does not fully express the same season fluctuation pattern as found at the number 2 sampling area, yet, the overall trend of seasonal population fluctuation as related to soil depth is indicated (Tables II and III).

In this study the field sampling revealed that P. brachyurus nematode is at its highest population level in the soil during the peanut harvest months of September, October, and November (Tables I and II). This confirms other research indications (28, 29, 38) that this is the best time of the year to take soil samples for the prediction of the following year's P. brachyurus nematode population. It is also indicated by this study that the soil depths most likely to have a detectable active population of the nematode is located in the 7.62 to 22.86 sampling zone.

Pratylenchus brachyurus nematode is reported to be sensitive to soil pH (18), hence soil pH was taken of samples thoughout the sampling of areas 2 and 3. The pH readings had a wide range of 4.76 to 7.75 and when the number of nematodes at each pH reading was placed in a graph, the results were a normal curve (Figure 5). Extremes were not connected to the line graph due to their infrequency which suggests the possibility of mechanical and human error. The largest number of P. brachyurus nematodes were recovered from soil samples with pH ranging from 5.6 to 6.25 which compares with the results reported by Willis (42).

The soil temperature readings recorded at each of the sampling

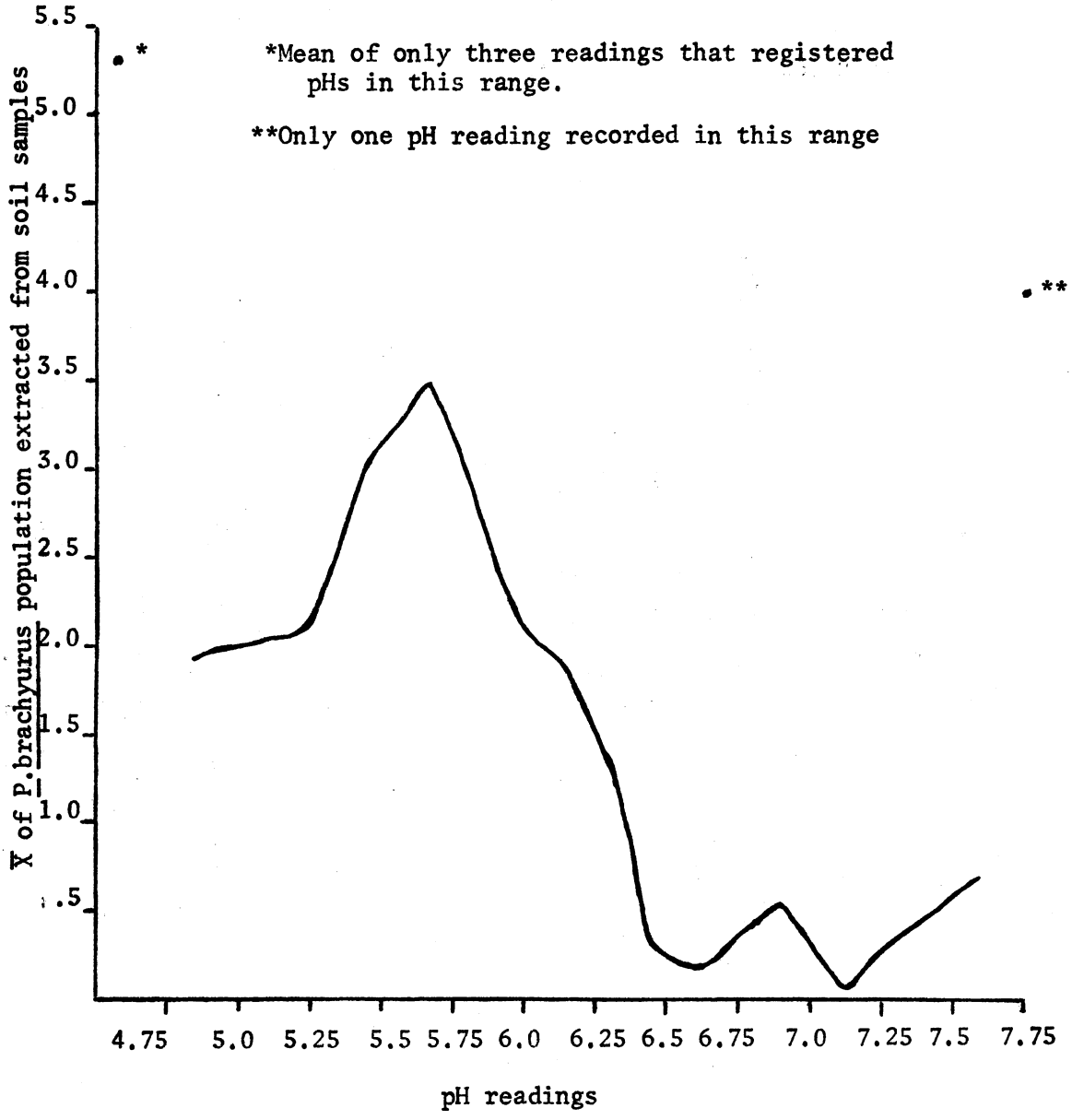

Figure 5. Mean of P. brachyurus Population Extracted from Soil with Various pH Levels

depths indicated nothing different from that previously observed by other researchers. Soil temperatures during the months June, July, and August appear to reach the levels that are reported to be inhibitory to Pratylenchus nematode spp. activity in the soil (3, 7, 10, 17). Soil temperatures during these months ranged from 25 C at the 30.48 to 38.10 cm depth to 34 C at the 0 to 7.62 cm depth (Figures 3 and 4). These temperatures are within, or very near to, the range of temperatures reported to reduce the mobility of Pratylenchus nematode spp. and this may be the major factor in P. brachyurus nematode being difficult to recover from soil samples taken during these months.

The greatest number of P. brachyurus nematodes were extracted from the soil samples during periods when the soil temperature was ranging between 15.6 and 26.7 C which occurred frequently during the months of September, October, and November (Figures 1 and 2). Also, there was a period during April, May, and early June that soil temperatures were within the range reported to favor the greatest amount of P. brachyurus nematode activity in the soil (3). A slight increase in the number of P. brachyurus nematodes extracted from the soil taken from area 3 was noted during these months. However, the number was so small that the nematodes could have been easily missed. The results obtained indicate that soil temperature is probably not the major factor causing P. brachyurus nematode to become active in the soil at population levels that can be detected during the spring of the year.

The overall results of the soil sampling study illustrates that even though a small number of P. brachyurus nematodes may be extracted from the soil at various sampling depths during all periods of the year, the possibility of missing the nematodes, even at the deeper soil

sampling depths, is so great that this type of sampling cannot be relied upon to make evaluation of the P. brachyurus nematodes present. Even during periods of the growing season when processed roots from peanut plants indicate detectable populations are present, the commonly used soil sampling and extraction techniques are still not an infallible means of finding the P. brachyurus nematode. This fact is indicated by Table V which presents the results obtained from roots of peanut plants taken from the sampling areas. The occurrence of subdetectable population levels appeared so often throughout this study that the results of the population counts could not be reliably evaluated by statistical methods. Therefore, any agricultural consultant who uses the results of soil samples to unequivocablly predict that P. brachyurus nematode is not present in a grower's peanut field may find himself in an embarrassing situation within the next crop year.

TABLE V

NUMBER OF P. BRACHYURUS NEMATODE EXTRACTED FROM ROOTS OF CROPS GROWING IN SAMPLING AREAS TWO AND THREE DURING SAMPLING PERIODS

Date	Area Two		Area Three	
	Host	# Nemas	Host	# Nemas
Jan.	Rye	No Sample	Rye	No Sample
Feb.	Rye	0	Rye	0
Mar.	Rye	0	Rye	0
Apr.	Rye	No Sample	Rye	No Sample
May 7	Rye	No Sample	Rye	No Sample
May 20	Planting Date		Planting Date	
June 12	Peanut	0	Peanut	0
June 20	Peanut	0	Peanut	0
July 15	Peanut	0	Peanut	52
July 31	Peanut	4	Peanut	4
Aug.	Peanut	4	Peanut	12
Sept.	Peanut	No Sample	Peanut	No Sample
Oct.	Peanut	56	Peanut	0
Nov.	Fallow	No Sample	Fallow	No Sample
Dec.	Rye	No Sample	Rye	No Sample

CHAPTER IV

OKRA BIO-ASSAY STUDY

The purpose of this study was to use a bio-assay as a means of checking the accuracy of the Modified Seinhorst Quick Extraction technique in detecting the presence of P. brachyurus nematodes in soil samples taken from Oklahoma peanut fields.

Methods and Materials

A series of bio-assays was used to check for the existence of P. brachyurus nematode in the soil samples having zero or very low populations of this nematode as analyzed by the Modified Seinhorst Quick Extraction technique (31). To establish the bio-assays, okra was used as the trap plant, and the plants were grown in 7.62 x 15.24 cm plastic pots containing 450 grams of soil previously analyzed. The soil used in bio-assays 1 and 2 was taken from samples collected from area 3 during the November and December sampling. The third bio-assay was established using soil taken during late season (post harvest) from several peanut fields located in Hughes and surrounding counties. In this bio-assay the soil samples were also taken at a depth of 7.62 to 22.86 cm with approximately 470 milliliters of soil collected at each site on a given sampling date and processed by the Modified Seinhorst Quick Extraction technique to determine the presence of P. brachyurus nematode.

In all 3 bio-assay studies, okra seed was allowed to germinate in

water for 5 days and at the end of this period, 5 seedlings were transplanted to each of the pots containing the field soil to be tested. The pots were placed in a controlled environmental growth chamber for 6 weeks at 23.9 $\pm$ 2 C day time temperature and 18.3 $\pm$ 2 C night time temperature. The pots were then removed from the growth chamber and the plants removed from the soil. The soil was again subjected to the Modified Seinhorst Quick Extraction technique and roots were removed from the plants and aeriated in beakers filled with water for 7 days. At the end of the aeriation period the water from around the roots was poured onto a 325-mesh seive and the screenings washed onto 2 Scottie tissues supported by a 30-mesh screen in a 10.5 x 7.5 cm plastic tub. The excess water was then decanted off after 24 hours and the nematodes poured into a watch glass for identification and counting.

Results and Discussion

The original soil counts of the November, 1973 sampling revealed a moderate (16 larvae) infestation of P. brachyurus nematode at the 7.62 to 15.24 cm, 15.24 to 22.86 cm, and the 30.48 to 38.10 cm sampling depths in area 3 (Table VI). However, when this soil was processed after a 35 day storage period, no P. brachyurus nematodes were extracted from soil taken from any sampling depth. When soil from the stored samples was subjected to a bio-assay using okra as the trap plant, counts ranging from 0 to 488 P. brachyurus nematodes were obtained from the okra roots and no P. brachyurus nematodes were recovered from the soil in which the okra had been growing (Table VI). Okra roots removed from the plants, grown in soil taken from the 7.64 to 15.24 cm sampling depth, produced a higher number of P. brachyurus nematode, significantly

TABLE VI

BIO-ASSAY - OKRA PLANTS GROWN IN SOIL
TAKEN FROM AREA 3 IN NOVEMBER, 1973

Bio-Assay #1 Depth	Soil Count After 35 Days In Storage	Rep 1 Root-Soil	Rep 2 Root-Soil	Rep 3 Root-Soil	Rep 4 Root-Soil	Rep 5 Root-Soil	Rep 6 Root-Soil	Rep 7 Root-Soil	Rep 8 Root-Soil	$\bar{x}$ of Total Root Counts [y]
0 - 7.62 cm	(0)*	72 (0)**	368 (0)	228 (0)	0 (0)	156 (0)	40 (0)	172 (0)	40 (0)	134.5 bc
7.62 - 15.24 cm	(0)	124 (0)	516 (4)	556 (0)	48 (0)	224 (0)	104 (0)	452 (0)	424 (8)	306.0 a
15.24 - 22.86 cm	(0)	64 (4)	135 (8)	216 (4)	156 (4)	150 (4)	260 (8)	264 (4)	260 (4)	188.3 b
22.86 - 30.48 cm	(0)	8 (0)	488 (0)	156 (0)	76 (0)	0 (0)	48 (0)	16 (0)	20 (0)	101.5 bc
30.48 - 38.10 cm	(0)	32 (0)	80 (0)	12 (0)	12 (0)	8 (0)	12 (0)	0 (0)	0 (0)	19.5 c

* = Soil counts recovered after storage for in laboratory at room temperature
** = Original soil counts of soil samples taken in November, 1973
y = All nematode counts were made from nemas extracted from okra roots grown in soil from each sampling depth. Data followed by the same letters in column within a sampling depth are not significantly different.
P = 0.05 according to Duncan's multiple range test.

different from the other sampling depths according to Duncan's Multiple Range (Table VI).

Analysis of the soil samples from area 3 collected in December, stored under the same conditions as the November sampling, produced no P. brachyurus nematodes prior to nor following the okra bio-assay although the original soil counts had revealed a trace infestation of P. brachyurus nematode (Table VII). As in the bio-assay of the November sampling, the largest number of nematodes was recovered from the roots grown in soil collected from the 7.62 to 15.24 cm sampling depth (Table VII). In both bio-assays the roots from okra plants grown at the various sampling depths produced counts of P. brachyurus nematode that were significantly greater than the original soil counts and the soil counts obtained just prior to planting the okra (Tables VI and VII).

The third bio-assay was set up using soil collected from several fields in Hughes and surrounding counties during the 1974 peanut growing season. The results of this bio-assay are presented in Table VIII. The results were generally the same as in the bio-assay involving soil from area 3. Again the roots grown in soil from the 7.62 to 15.24 cm sampling depth produced the highest number of P. brachyurus nematodes.

Even though the soil sample counts indicated that the active population of P. brachyurus nematode is generally found at the deeper sampling depths during the winter months, the bio-assay demonstrated the soil collected from the upper sampling depths contained inactive life stages that are necessary to produce high root populations of P. brachyurus nematode. The bio-assay indicates that the soil containing the highest number of inactive life stages is located in the range between the 7.62 to 15.24 cm sampling depth. It is indicated that soil

TABLE VII

BIO-ASSAY - DETECTION OF P. BRACHYURUS NEMATODE UTILIZING OKRA PLANTS GROWN IN SOIL TAKEN FROM AREA 3 DURING DECEMBER, 1973

Bio-assay #2 Depth	Soil Counts After 35 Days In Storage	Rep 1		Rep 2		Rep 3		Rep 4		X of Total Root Counts[y]	
		Root	Soil**	Root	Soil	Root	Soil	Root	Soil		
0 - 7.62 cm	(0)*	0	(0)**	40	(0)	156	(4)	0	(0)	49	b
7.62 - 15.24 cm	(0)	292	(0)	474	(0)	328	(0)	640	(0)	433	a
15.24 - 22.86 cm	(0)	0	(8)	12	(0)	250	(0)	0	(0)	68	b
22.86 - 30.48 cm	(0)	0	(0)	0	(4)	16	(4)	48	(0)	16	b
30.48 - 38.10 cm	(0)	0	(0)	0	(0)	0	(4)	4	(0)	1	b

* = Soil counts recovered after storage for 35 days in laboratory at 21.1 C
** = Original soil counts of soil samples taken in December, 1973
y = All nematode counts were made from nemas extracted from okra roots grown in soil from each sampling depth
Data followed by the same letters in column within a sampling depth are not significantly different.
P = 0.05 according to Duncan's Multiple Range test.

TABLE VIII

BIO-ASSAY - DETECTION OF P. BRACHYURUS NEMATODE UTILIZING OKRA PLANTS GROWN IN SOIL TAKEN FROM FIELDS IN HUGHES COUNTY DURING NOVEMBER, 1974

Depth	Number of Samples From Each Depth	Total Soil Count*	Total Root Count
0 - 7.62 cm	2	0	40
7.62 - 15.24 cm	4	0	628
15.24 - 22.86 cm	3	4	40
22.86 - 30.48 cm	2	0	0
30.48 - 38.10 cm	1	0	0

* = Original soil counts taken in November, 1974 sampling.

from all sampling depths contains the inactive life stages of P. brachyurus nematode that are necessary to produce a high infestation on roots of susceptible plants, but the area in the soil where the highest concentration of peanut pegs, pods, and small feeder roots are located also has the highest number of inactive P. brachyurus nematode life stages. This results in higher numbers of P. brachyurus nematode infesting the roots of the susceptible okra plants grown in the soil taken from the sampling depth of 7.62 to 15.24 cm. Although all sampling levels were shown to have the potential to produce moderate to high populations of P. brachyurus nematode the potential for higher populations becomes greater in the upper horizons of the soil. The exception to this observation is the 0 to 7.62 cm sampling depth which has the potential to produce a high population but it appears not to contain as high a concentration of inactive life stages as the soil lying between the 15.24 to 22.86 cm range.

The results of the bio-assays also support the observation made in the field sampling study by illustrating that soil processed by commonly used nematode extraction techniques and shown to have no or very low P. brachyurus nematode populations often contains inactive or immobile life stages of the nematode. These stages can be stimulated to activity and infestation of the roots of a susceptible plant by growing the plant in soil in which the inactive life stages are present.

The okra bio-assay proved to be a more efficient and dependable method of detecting P. brachyurus nematodes in soil collected during periods of the year when standard nematode extraction procedures fail to detect the nematode. Therefore it is submitted that a bio-assay can be used as an effective tool to evaluate or replace standard nematode

extraction techniques when the worker is interested in detecting P. brachyurus nematode in the soil of Oklahoma fields. However, the bio-assay has the disadvantages of requiring 4 to 6 weeks before results can be obtained. Also, if a large number of samples are being processed the space and time required for growth and maintenance of the trap plant is prohibitive.

CHAPTER V

EXPERIMENTAL NEMATODE CULTURING AND EXTRACTING TECHNIQUES

Three experimental techniques were evaluated as methods that would stimulate P. brachyurus nematode to become active in soil and increase chances of detecting its presence. It was hoped that a procedure could be developed which would allow quick and accurate analysis of soil for determining the presence of P. brachyurus nematode.

Methods and Materials

A soil test designed on work reported by Meagher (19) of alternating wetting and drying periods was carried out to provide a stimulus that would cause the root-lesion nematode, P. brachyurus, to become active in soil samples collected during the late winter and early spring months.

This test consisted of a 100 milliliter alliquot of soil placed on 4 Scottie tissues supported by a 30-mesh screen in a 7.5 cm x 10.50 cm plastic tub. The soil samples were alternately saturated with water and then allowed to dry by allowing the water level in the tub to evaporate to the point where it was no longer in contact with the soil and, when the soil no longer contained noticeable moisture, the water level in the tub was raised and the soil again saturated. This procedure was repeated, if required, at the following time intervals: (1) 36 hours,

(II) 84 hours, (III) 96 hours, (IV) 120 hours, (V) 8 days, (VI) 3 weeks, and (VII) 4 weeks. At the end of each period the Scottie tissues and soil were transferred to a new tub containing a water level high enough to resaturate the soil. Water from the original tub was then decanted and the remaining portion was poured into a watch glass for identification and counting of any nematodes recovered.

At the same time as the wetting and drying test was being conducted, a heat stimulation test was conducted in an attempt to cause P. brachyurus nematode to revert from its apparent arrested development stages and become active in the soil in a mobile life stage which could be extracted by commonly used extraction techniques. The soil was subjected to different time periods of a temperature reported to favor the increased activity of Pratylenchus nematode spp. (25).

In this test 16 non-vented polyethylene bags were filled with a 100 milliliter alliquot of soil and placed in a laboratory culture chamber at 24.8 C and the samples removed at different time periods. The soil used in this study was taken from the same samples that were subjected to the bio-assay test and known to have a moderate infestation of P. bracyurus nematode, based on the counts obtained from the original soil samples taken in November.

Two bags of soil were removed from the culture chamber at each of the following time intervals, which were considered as treatments: (I) 0 hours, (II) 36 hours, (III) 48 hours, (IV) 60 hours, (V) 72 hours, (VI) 84 hours, (VII) 96 hours, and (VIII) 144 hours. The soil used in this test was collected in November, 1973 from sampling area 3 at depths of 15.24 to 30.48 cm, having a field soil temperature ranging between 15.0 C to 17.3 C and stored at 20 $\pm$ 2 C for 35 days prior to this test.

In addition to above described experiments, a raw, root exudate test was carried out to determine if germinating okra and peanut seed produced diffusates that would stimulate P. brachyurus nematode to become active in the soil so that they could be extracted and identified by commonly used nematode extracting techniques.

Fifty okra seeds were allowed to germinate in a beaker containing 50 milliliters of water, and 20 peanut seeds were germinated in a separate beaker containing 100 milliliters of water. A 100 milliter alliquot of soil was taken from each soil sample that was being subjected to the root exudate experiment and the remainder of the soil sample was subjected to a bio-assay test. The okra seeds were allowed to germinate in the water for 5 days and as the radicals reached an average length of 1.0 centimeter the seedlings were removed and the water used to make up the different treatments in this experiment. These treatments consisted of (1) two 7.62 cm x 10.60 cx pots containing soil receiving 10 milliliters each of the water used for germinating the okra seed, (II) 2 pots receiving 10 milliliters each of a 1:1 ratio of germination water and distilled water, (III) 2 pots receiving 10 milliliters each of a 1:10 ratio that was 1 part germination water to 10 parts distilled water.

To further evaluate for root exudates, the 20 percent seeds were allowed to germinate in distilled water for 4 days, then removed and the radicals clipped and weighed. The radicals were then pulverized, using a mortor and pestle, and the pulp was strained through a cheese cloth filter and the liquid collected. Twelve pots, the same size as used in the okra test, were filled with 100 milliliters of the stock soil and treated as follows: (I) 4 pots were treated with 10 milliliters each of

1 part extract to 0 parts distilled water, (II) 4 pots were treated with 10 milliliters each of the dilution of 1 part extract to 1 part distilled water, and (III) 4 pots were treated with 10 milliliters each of the dilution of 1 part extract to 10 parts distilled water. All treatments in the root exudate test were maintained in the environmental growth chamber at 23.9 $\pm$ 2 C for 2 weeks prior to being analyzed by the Modified Seinhorst Quick Extraction technique.

Results and Discussion

The Wet - Dry Cycle and the Heat Stimulation experiments were carried out at the same time as the first bio-assay. These tests were established to determine the effect of different periods of exposure to a set temperature and soil moisture levels. All treatments in both tests failed to stimulate P. brachyurus nematode activity as can be observed by the results presented in Tables IX and X. The soil used in both of the tests was from the same stock soil as used in the first bio-assay, which proved the presence of P. brachyurus nematodes in the soil by producing root counts that ranged from 4 to 424 (Table VI).

The raw exudates from both the okra and peanut roots did not stimulate activity of P. brachyurus nematode. This test was conducted with the hope of producing a similar nematode attracting effect as that reported being produced by nematode trapping fungi (23). However, in all cases, both the okra and peanut root exudates did not appear to stimulate P. brachyurus nematode to terminate its state of inactivity. At the same time, the okra bio-assay produced P. brachyurus nematode counts that ranged from 72 to 424 (Table XI).

This test was designed to evaluate a simple method of collecting

TABLE IX

WET - DRY CYCLE TEST

COUNTS OF P. BRACHYURUS NEMATODE OBTAINED
AFTER EACH TREATMENT PERIOD

Time Period	Depth*	Rep	Nematode Count After Treatment	Bio-assay Counts**
36 hrs	7.62 - 15.24 cm	2	0	224
84 hrs	7.62 - 15.24 cm	2	0	424
96 hrs	15.24 - 22.86 cm	2	0	156
120 hrs	22.86 - 30.48 cm	2	0	76
8 days	15.24 - 22.86 cm	2	0	156
3 wks	7.62 - 15.24 cm	2	0	124
3 wks	22.86 - 30.48 cm	1	0	76
4 wks	15.24 - 22.86 cm	3	0	156

* = Sampling depth from which soil was taken for treatment.

** = Number of P. brachyurus extracted from okra roots grown in soil from the same depth as the soil involved in the test.

TABLE X

HEAT STIMULATION TEST

TO PRODUCE ACTIVE SOIL POPULATIONS
OF P. BRACHYURUS NEMATODE

Treatment	Number of Samples	Number of Nematodes Recovered	Bio-assay Counts*
0 hrs	2	0	72
36 hrs	2	0	40
60 hrs	2	0	0
72 hrs	2	0	64
84 hrs	2	0	104
96 hrs	2	0	216
144 hrs	2	0	424

* = Number of P. brachyurus nematode extracted from okra roots grown in soil from the same depth as the soil involved in corresponding time period.

TABLE XI

GERMINATION - DILUTION TEST

TO EVALUATE RAW ROOT EXUDATE EFFECTS ON SOIL BEING ANALYZED FOR P. BRACHYURUS NEMATODE

Peanut root diffusate			
Dilution	Reps	Post-Treatment Counts**	Bio-Assay Counts***
Undiluted (4)*	4	0	10
1:1 (0)	4	0	94
1:10 (0)	4	0	12
Okra root diffusates			
Dilution	Reps	Post-Treatment Counts**	Bio-Assay Counts***
Undiluted (28)	2	28	72
1:1 (12)	2	12	424
1:10 (20)	2	8	162

* = number of nemas recovered from soil before treatment with root diffusates or bio-assay.

** = number of nemas extracted from 150 ml alliquot of soil 21 to 28 days after treatment with root diffusates.

*** = number of P. brachyurus recovered from okra plant roots planted in same soil used for treatments with root diffusates.

root exudates from germinating peanut and okra seeds in an attempt to develop an expedient method of determining the presence of P. brachyurus nematode in collected and/or stored soil samples. This test proved to be unsuccessful and no evaluation could be made on the test results. The bio-assay illustrated that the presence of a growing okra plant in the soil stimulated P. brachyurus nematode to become active and invade the plant roots, and it has been reported that nematodes cause a change in the exudates produced by infested roots (24, 25). Therefore, it becomes reasonable to assume that the plant roots are producing a diffusate which is a stimulus and an attractant to the P. brachyurus nematode. A bio-chemical reaction is indicated and could be useful in developing a new procedure that would be faster and more proficient in detecting inactive or dormant nematode populations in the soil.

CHAPTER VI

SUMMARY

1. Monthly sampling showed September, October, and November to be the months with the highest overall soil population of P. brachyurus nematode.

2. Sampling indicated some slight activity of P. brachyurus nematode in the soil during April, May, and June.

3. Soil populations of P. brachyurus nematode during all months, except September, October, and November, may be so low that they can be easily missed by current or standard soil sampling procedures and nematode extraction techniques.

4. The study indicated that the lower sampling depths might support a small active P. brachyurus nematode population throughout the entire year.

5. Top soil temperatures occurring in Oklahoma appeared to directly affect the activity of P. brachyurus nematode and in the winter, the soil temperatures were well below the range at which this nematode is normally active.

6. Sampling depth required for the recovery of the largest numbers of P. brachyurus nematode appears to be between 15.24 and 30.48 cm. This was the soil depth found to contain the largest percentage of the year's total population. Therefore, it remains the recommendation of Oklahoma State University agriculture advisory personnel that sampling

for populations of P. brachyurus in Oklahoma peanut fields should be done during the harvest months of September, October, and November at depths between 15.24 cm and 30.48 cm.

7. Largest percent of each month's P. brachyurus nematode population fluctuates up and down in the soil. Months with severe temperatures, either low or high, appear to have a larger percent of the month's total population at the lower sampling depths.

8. Bio-assays proved that standard soil sampling and nematode extracting techniques could not be fully relied upon to detect P. brachyurus nematode infestations in the spring, summer, and winter months.

9. The heat stimulation and alternating dry and wet cycle indicated that manipulating these soil conditions will not stimulate the activation of P. brachyurus nematode in soil samples collected during the winter.

10. The germination dilution tests illustrated that a sample method of collecting raw root exudates did not succeed and that the growing of okra plants in soil suspected of being infested with P. brachyurus nematode provides a stronger stimulus than that provided by the collected exudates.

LITERATURE CITED

1. Baermann, G. 1917. Eine enifache methode zue affindung von Aklyostomum (Nematoden) larven in erdrpoben. Geneesk. Trijdschr. Nederl. Indie 57:131-137.

2. Boyle, Lytton W. 1950. Several species of parasitic nematodes on peanuts in Georgia. Plant Dis. Rep. 34:61-62.

3. Brodie, B. B. and B. H. Quattlebaum. 1970. Vertical distribution and population fluctuations of three nematode species as correlated with soil temperature, moisture, and texture. Phytopathology 60:1286 (Abstr.).

4. Caveness, F. E. and H. J. Jensen. 1955. Modification of the centrifugal flotation technique for the isolation and concentration of nematodes and their eggs from the soil and plant tissue. Pro. Helminth. Soc. Was. 22(2):87-89.

5. Christie, H. R. and V. G. Perry. 1951. Removing nematodes from soil. Proc. Helminth. Soc. Wash. 18:106-108.

6. Collins, R. J. and R. Rodriguez-Kabana. 1971. Relationship of fertilizer treatments and crop sequence to populations of lesion nematodes. J. Nematol. 3:306-307 (Abstr.).

7. Di Edwardo, A. A. 1961. Seasonal population variations of Pratylenchus penetrans in and about strawberry roots. Plant Dis. Rep. 45:67-71.

8. Dunn, R. A. 1972. Importance of depth in soil, presence of host roots, and role of eggs as compared to veriform stages in overwintering of Pratylenchus penetrans at Ithaca, New York. J. Nematol. 4:221-222 (Abstr.).

9. Endo, Burton Y. 1959. Responses of root-lesion nematodes, Pratylenchus brachyurus and P. zeae to various plants and soil types. Phytopathology 49:417-421.

10. Feldmesser, J. and R. V. Rebois. 1965. Temperature and moisture effects on Pratylenchus brachyurus. Nematologica 11:37-38 (Abstr.).

11. Good, J. M., W. K. Roberson, and L. G. Thompson. 1954. Effects of crop rotation on the populations of meadow nematodes, Pratylenchus leiocephalus, in Norfolk Loamy Fine Sand. Plant Dis. Rep. 38:178-180.

12. _____, L. W. Boyle, and R. O. Hammons. 1958. Studies of Pratylenchus brachyurus on peanuts. Phytopathology 48:530-535.

13. _____. 1962. Fertilizers can transmit plant nematodes. Phytopathology 52:11 (Abstr.).

14. _____ and J. R. Stansell. 1965. Effect of irrigation, soil fumigation, and date of harvest on Pratylenchus brachyurus (Godfrey) infection on pegs, peg rot and yield of peanuts. Nematologica 11:38 (Abstr.).

15. Jenkins, W. R. 1964. A rapid centrifugal flotation technique for separating nematodes from soil. Plant Dis. Rep. 41:692.

16. Kable, P. F. and W. F. Mai. 1968. Influence of soil moisture on Pratylenchus penetrans. Nematologica 14:101-122.

17. Kerr, A. and M. K. Vythilingam. 1966. Factors influencing the extraction of nematodes from soil. Nematologica 12:511-517.

18. Koen, H. 1967. Notes on the host range, ecology and population dynamics of Pratylenchus brachyurus. Nematologica 13:118-124.

19. Meagher, J. W. 1970. Seasonal fluctuations in numbers of larvae of the cereal cyst nematode (Heterodera avenae) and of Pratylenchus minyus and Tylenchorhynchus brevidens in soil. Nematologica 16:333-347.

20. Miller, Patrick M. 1957. A Method for the quick separation of nematodes from soil samples. Plant Dis. Rep. 41:194.

21. Minton, N. A., R. O. Hammons, and S. A. Parham. 1970. Infection of shell and peg tissues of six peanut cultivars by Pratylenchus brachyurus. Phytopathology 60:472-474.

22. _____ and H. B. Gillenwater. 1973. Methyl bromide fumigation of Pratylenchus brachyurus in peanut shells. J. Nematol. 5:147-149.

23. Monoson, Herbert L., Alan G. Galsky, Judith A. Griffin, and Eleanor J. McGrath. 1973. Evidence for a partial characterization of a nematode attraction substance. Mycologia 65:78-86.

24. Morrison, L. S. 1975. Personal Communication.

25. Patterson, Sister Mary Thomasine and G. B. Bergeson. 1967. Influence of temperature, photoperiod, and nutrition on reproduction, male-female juvenile ratio and root to soil migration of Pratylenchus penetrans. Plant Dis. Rep. 51:78-82.

26. Powell, William M. and C. J. Nausbaum. 1963. Investigation on the estimation of plant parasitic nematode population for advisory purposes. North Carolina Agr. Exp. St. Tech. Bull. 156. 22p.

27. Radewald, John D. and Glenn Takeshita. 1964. Desiccation studies on five species of plant-parasitic nematodes in Hawaii. Phytopathology 54:903 (Abstr.).

28. Russell, C. C., C. G. Shackelford, and L. S. Morrison. 1972. Unpublished Report to Oklahoma Peanut Commission.

29. _____. 1975. Personal Communication.

30. Seinhorst, J. W. 1956. Een eeNovoudige method voor het afsheiden van aaltjes uit ground. Tijdschr. Piziekt 61(6):188-190.

31. _____. 1956. The quantative extraction of nematodes from soil. Nematologica 1:249-267.

32. Sher, S. A. and M. W. Allen. 1953. Revision of the genus Pratylenchus (Nematode: Tylenchidae). Calif. Univ. Publication Zoo. 57:441-470.

33. Southey, J. F. (Editor). 1959. Plant Nematology. Pratylenchus spp. and other migratory soil nematodes. By R. S. Pritcher. pp. 77-87.

34. Steiner, G. 1942. Plant nematodes the grower should know. Soil Sci. Society Flor. Proc. IV;72-117.

35. _____. 1945. Meadow nematodes as the cause of root destruction. Phytopathology 35:935-937.

36. Struble, F. B., L. S. Morrison, L. A. Brinkerhoff, H. C. Young and H. B. Cordner. 1960. Unpublished Annual Progress Report for Project Hatch 941. On file, Oklahoma State University Plant Pathology Department.

37. Taylor, A. L. 1953. Nematodes associated with root-lesion of Abaca. Turrialba 3:8-15.

38. Thames, Walt. 1975. Personal Communication.

39. Thorne, G. 1961. Principles of Nematology. McGraw and Hill, New York. 522p.

40. Tripp, L. D. 1975. Personal Communication.

41. Wang, Ernie L. H. and G. B. Bergeson. 1974. Biochemical changes in root exudate and xylem sap of tomato plants infected with Meloidogyne incognita. J. Nematol 6:194-202.

42. Willis, C. B. 1972. Effects of soil pH on reproduction of Pratylenchus penetrans and forage yield of alfalfa. J. Nematol. 4: 291-295.

43. Young, T. W. 1954. An incubation method for collecting migratory endo-parasitic nematodes. Plant Dis. Rep. 38:794-795.

VITA

Phillip Wade Pratt

Candidate for the Degree of

Master of Science

Thesis: DETECTION OF SEASONAL PRATYLENCHUS BRACHYURUS NEMATODE POPULATION

Major Field: Plant Pathology

Biographical:

Personal Data: Born in Elk City, Oklahoma, January 24, 1949, the son of Wade and Blanche Pratt.

Education: Attended grade school at Butler Elementary School, Butler, Oklahoma; graduated from Butler High School, Butler, Oklahoma, in 1967; received Bachelor of Science Degree in Botany and Plant Pathology from Oklahoma State University, College of Agriculture, Stillwater, Oklahoma, in July, 1971; completed requirements for the Master of Science degree from Oklahoma State University in May, 1977.

Professional Experience: Graduate Research Assistant, Department of Plant Pathology, Oklahoma State University, 1971-1975.